Variability of House Dust Mite Allergen Exposure in Dwellings

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The variability of repeated house dust mite (HDM) allergen determinations at the same site within 3–24 months was evaluated on previously collected samples. Between two and four repeated measurements of Der p 1, a major allergen of *Dermatophagoides pteronyssinus* and Der f 1, a major allergen of *D. farinae*, on 46 carpets and 31 mattresses were analyzed. In 90% of carpets and mattresses, HDM allergen concentrations were clinically relevant (at least one measurement >0.1 µg Der p 1 + Der f 1/g dust). The coefficients of variation (CVs) for allergen concentrations in repeated samples over time (55.3–82.0% for the two allergens in beds and carpets) were clearly greater than the CVs for multiple samples collected at the same time (4.0–32.6%). Determination of allergen mass per square meter of surface instead of concentration per gram of dust resulted in an even greater CV (72.3–86.7%). The 95% range of expected values was about 10-fold above and below the result of a single determination. We conclude that single determinations of HDM allergen in dust give very limited information about long-term exposure of an individual to the allergen. Repeated measurements are recommended. Studies of factors that affect HDM allergen exposure must be planned with appropriate sample sizes. *Key words*: asthma, house dust mite allergens, indoor allergen exposure, reliability, repeated determination, sample size, variability. *Environ Health Perspect* 106:659–664 (1998). [Online 11 September 1998] *http://ehpnet1.niehs.nih.gov/docs/1998/106p659-664hirsch/abstract.html*

Some studies have shown that exposure to house dust mite (HDM) allergens at home is associated with prevalence of allergic sensitization (*1–3*) and asthma (*4,5*) in susceptible children, but others did not (*6,7*). The reliability of the determination of indoor exposure to these allergens has not been investigated extensively. Marks and co-workers (*8*) calculated that threefold above and below a result was the range within which the true value lies with 95% certainty. The authors referred to 117 duplicate determinations of Der p 1, a major allergen of *Dermatophagoides pteronyssinus*, within 2 weeks in Sydney, Australia.

In contrast to most other parts of the world (*2,3,6,9*), Sydney is characterized by extremely high concentrations of Der p 1 [geometric mean 38.9 µg/g dust on mattresses and 22.4 µg/g dust on bedroom floors (*8*)] and the absence of other HDM allergens. Two recent studies have shown that the prevalence of allergic sensitization in children correlates with exposure to HDM allergen at concentrations far below these levels [0.1 µg Der p 1 + Der f 1 (a major allergen of *D. farinae*)/g carpet dust (*3*) or even lower (*2*)].

Thus, it seems reasonable to compare the Australian results with data from a European region with low to moderate levels of Der p 1 and, in addition, moderate concentrations of the HDM allergen Der f 1 to answer two questions: 1) Is the (relative) variability of allergen concentration higher at lower exposure levels? 2) Is the variability of Der f 1 exposure lower than that of Der p 1 because of the greater resistance of *D. farinae* to changes in humidity and temperature (*10–12*)?

There are some other considerations of practical relevance that have not been addressed so far. First, the Australian study measured variability in 2-week intervals. This design might fail to detect long-term variations of exposure. Second, the allergen concentration in sampled dust might be massively "diluted" with allergen-free dirt from outside. Is the total sampled mass of allergen a measure of less variability? Finally, can the reliability be improved if sampling is always done by the same trained field worker rather than by residents following written instructions?

Answers to these questions should help improve methods of future field studies of HDM allergen exposure. For this purpose it is also useful to estimate how many samples are needed to compensate for the variability caused by influences that cannot easily be controlled by study design.

Methods

Study Design

Variability of sampling and dust extraction procedures. To describe the real variability of HDM exposure, it is necessary to assess the impact of laboratory procedures on total variability. Besides the variability of the assay itself (interassay and intra-assay variability; see "Laboratory Analysis") the influence of our sampling and dust extraction procedures on the variability of results was examined in a methodical study.

Sample pairs from six mattresses (both longitudinal halves) and six carpets (two squares of 1 m^2) were collected at the same time. To evaluate the variability of the extraction procedure, each of these two samples and two additional single samples from two mattresses (n = 26) were divided into two to nine dust portions. These portions were analyzed separately, and the mean and the coefficient of variation (CV) were calculated for each set of portions. The mean was interpreted as the value of the whole original sample. The values of the two original samples collected at one site were then used to calculate CVs for sampling at the same site at the same time.

Long-term variability of allergen concentration at the same site. We retrospectively analyzed dust samples from two field studies in which HDM allergen exposure was determined repeatedly (within 3–24 months). Study 1 was a study of immunotherapy (December 1993–May 1996, n = 28 mite allergic children), during which dust samples were taken from the carpets in the children's bedrooms and their mattresses at month 0, 3, 12, and 24 of the study. Study 2 was a cohort study (December 1994–February 1996, n = 101 non–mite-allergic infants), during which samples were acquired from mattresses and bedroom carpets within 4 weeks after the probands' birth and at the age of 6 months. Both studies were conducted after informed consent was obtained from all participants' parents for all study elements including analysis of house dust for indoor allergens.

The residents were asked whether they had taken actions that might affect allergen exposure (change of residence, change of carpets or bedding, encasing of mattresses or pillows). In case they had done so, samples before and after the interventions were not compared in our analysis. In addition, samples taken between August and November, a season with higher exposure (*13,14*), were excluded because season is a known cause of variability and can be controlled in the design and evaluation of studies of HDM allergen exposure. Moreover, this standardization improves the comparability of the

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individual cases and makes the study comparable to the Australian study, which did not measure seasonal effects.

These exclusion criteria and a number of study dropouts and samples with insufficient amount of dust (mainly from babies' mattresses) reduced the number of dust samples theoretically available (101 doubles and 28 quadruples from carpets and mattresses). In all, 46 sets of repeated measurements in carpets (28 doubles, 8 triples, 10 quadruples) and 31 sets of repeated measurements on mattresses (20 doubles, 6 triples, 5 quadruples) were analyzed.

Sampling Procedure

Carpets (1 m^2) and children's mattresses (1.6 m^2, covers removed) were vacuumed for 5 min with vacuum cleaners with a motor capacity of at least 800 W by residents following written instructions (study 1) or by trained field workers (study 2). Dust was collected with a sampling device (ALK, Copenhagen, Denmark) holding a cellulose filter (Filtrak GmbH Niederschlag, Germany, no. 60; 0.35 mm thick, pore size 30–40 µm), which retains 98% of particles >0.7 µm in diameter and 91% of particles >0.3–0.7 µm.

Laboratory Analysis

Samples were weighed and processed unsieved (allergen extraction from dust and filter paper) to allow the determination of total allergen amount. The concentration of HDM allergens Der p 1 and Der f 1 was determined with a commercial sandwich ELISA (ALK). The results were expressed as allergen concentration (microgram per gram of dust) and as amount of surface allergen (microgram per square meter). The intra-assay CV determined by the manufacturer was 4.1–19.9%. The interassay CV determined in our laboratory by positive control samples on every plate ranged between 9.2% and 16.6% (Der p 1) and 14.9% and 16.5% (Der f 1). Assay sensitivity was 3 ng/ml or 0.03 µg/g dust.

Statistics

The variability of the measurement of allergen concentrations in our studies was assessed by calculation of the variance observed for repeated measurements at the same site. This variance was related to the variance observed between different sites to assess its relative magnitude. The calculation and analysis of variances (ANOVA) are based on two main assumptions: the values are normally distributed, and the variances are the result of random effects.

We therefore proceeded in five steps: 1) assessment of the distribution of the measured values; 2) transformation of the values into a scale in which they were normally distributed; 3) exclusion of systematic effects on the variability; 4) quantification of the variability; and 5) retransformation of the results into the scale of the measurement.

Descriptive statistics showed a positively skewed distribution of values for concentration as well as for mass values (both mite allergens, carpet, and mattress). After transformation into $\log_{10}$ values, the distributions were not significantly different from normal (Shapiro–Wilks test).

The variability of repeated measurements did not depend on the mean of these measurements. Figure 1 shows the data for Der p 1. The plot for Der f 1 was similar (not shown). The sequence of the measurements at one site did not affect the results. This means, for example, that the first sampling procedure did not influence the results of the following procedures systematically (analysis of correlation coefficients; data not shown). We therefore assume that the results of single measurements were independent from each other (random effects model).

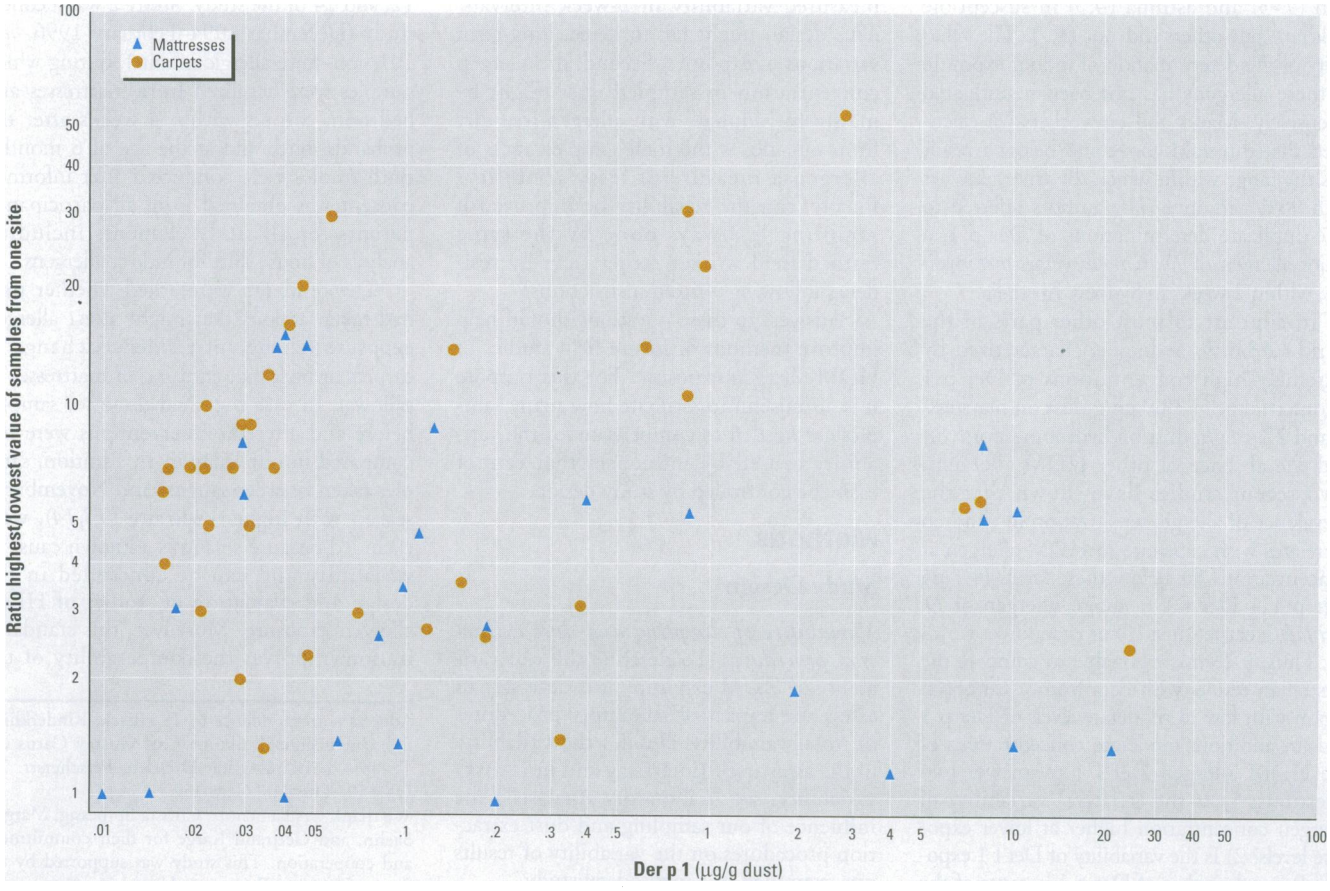

Figure 1. Der p 1 concentrations in repeated dust samples at one site within 3–24 months (geometric mean) and fold difference between the highest and the lowest value of these samples.

The $\log_{10}$ values were used in an ANOVA to calculate the variances between and within sites. Based on this ANOVA, we determined the single determination range (SDR) (*15*): the square root of the within-site variance was multiplied by 1.96 and re-transformed as the antilog into the nonlogarithmic scale.

$$SDR = 10^{\sqrt{\text{within site variance}}(1.96)}$$

The SDR indicates the range, in fold units, above and below a measured value, within which the true value can be expected in about 95% of the cases. Based on the SDR we calculated the 95% confidence interval (CI) for the mean of n measurements as a range, in fold units, above and below the mean by dividing the SDR by the square root of n. Vice versa, the number of samples necessary to reach a 95% CI of a given range (determined by the factor d by which the mean is multiplied or divided to determine the upper and lower limit) was calculated by

$$n = \frac{1.96(SDR^2)}{d^2}$$

The reliability of the measurements is calculated in the ANOVA as the ratio of between-measurement variance and total variance multiplied by 100. The smaller the variability at one site (the greater the correlation between repeated measurements), the closer the reliability is to 100%.

Sample sizes were determined for the *t*-test (two independent groups of samples) and the paired *t*-test (paired samples) using given mean differences and the calculated variance components in tables for the two-tailed *t*-test with a significance level of $\alpha = 0.05$ and a power of $1 - \beta = 0.8$ to detect a given effect (*16*).

The significance of the differences in the CVs in study 1 and study 2 was tested with the Mann Whitney *U*-test (significance level $\alpha = 0.05$).

Software for all calculations was SPSS (Chicago, IL) for Microsoft Windows 6.01.

To allow transformation of all data into the logarithmic scale, calculation values below the sensitivity threshold of 0.03 µg/g were estimated uniformly with 0.01 µg/g.

Results

Variability of Sampling and Dust Extraction Procedures

Allergen concentrations (median; range in micrograms per gram of dust) in the method evaluation study were 0.02 (0.01–8.61) for Der p 1 and 0.48 (0.01–7.67) for Der f 1 for carpet samples, and 30.71 (0.23–78.46) for Der p 1 and 11.06 (5.95–69.64) for Der f 1 for mattress samples. Median CVs for the

Table 1. Median and ranges of allergen exposure and coefficients of variation (CV) for house dust mite allergen measurements at the same site (field studies)

	Allergen exposure	Within-site CV (%)[a]
Carpets (n = 46; 120 samples)		
Der p 1 concentration (µg/g dust)	0.05 (<0.03–37.80)	82.0 (0–132.3)
Der f 1 concentration (µg/g dust)	0.18 (<0.03–41.38)	77.6 (0–140.5)
Der p 1 mass (µg/m^2)	0.02 (<0.003–31.00)	86.7 (5.2–149.6)
Der f 1 mass (µg/m^2)	0.05 (<0.003–10.35)	72.3 (9.6 –137.9)
Mattresses (n = 31; 78 samples)		
Der p 1 concentration (µg/g dust)	0.08 (<0.03–27.05)	55.3 (0–123.8)
Der f 1 concentration (µg/g dust)	1.32 (<0.03–205.00)	75.0 (0–189.0)
Der p 1 mass (µg/1.6 m^2)	0.05 (<0.003–45.99)	75.7 (14.1–131.2)
Der f 1 mass (µg/1.6 m^2)	0.47 (<0.003–171.73)	74.6 (6.9–194.5)

Der p 1, allergen of *Dermatophagoides pteronyssinus*; Der f 1, allergen of *D. farinae*.
[a]Calculation of CVs is based on nonlogarithmic data for concentration (µg/g dust) and mass (µg/m^2).

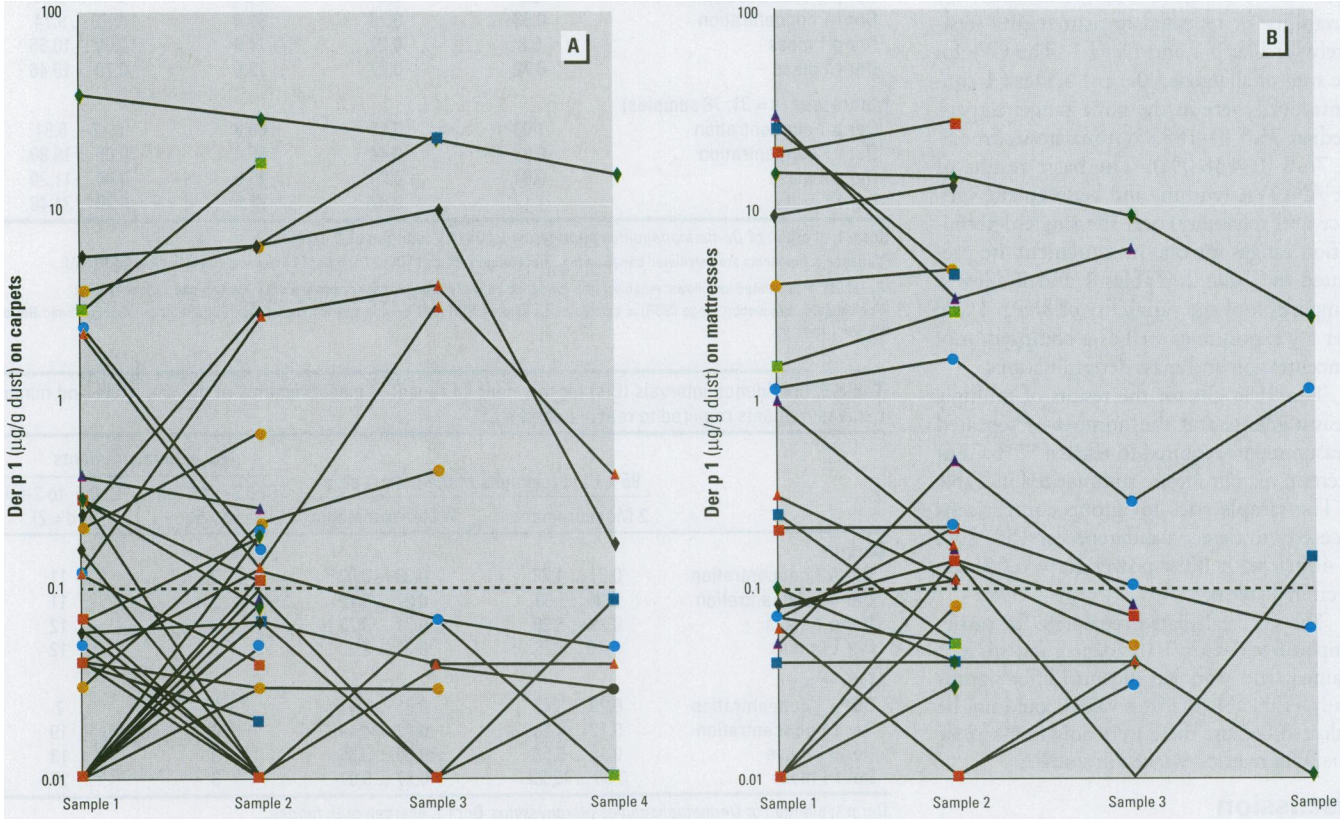

Figure 2. Variability of Der p 1 concentrations in two to four dust samples collected from (A) one carpet and (B) one mattress within 3–24 months. Solid lines connect results from the same site. The dashed lines represent the presumably clinically relevant concentration of 0.1 µg/g dust.

dust extraction procedure were 23.7% for Der p 1 and 13.9% for Der f 1 for carpet samples, and 11.9% for Der p 1 and 12.2% for Der f 1 for mattress samples. Median CVs for two samples from one site were 32.6% for Der p 1 and 7.9% for Der f 1 for carpet samples, and 4.0% for Der p 1 and 12.3% for Der f 1 for mattress samples. The CVs for allergen mass determination were in the same range (data not shown).

Long-term Variability of Allergen Concentration at the Same Site

The concentrations and masses of allergens detected in our field studies were in the same range as in the studies evaluating sampling and extraction variability, except the values for Der p 1 on mattresses were lower in the field studies (Table 1). Corresponding to the data for single allergens, the sum of allergen concentrations (Der p 1 + Der f 1/g dust) was markedly lower on carpets (median 0.35; maximum 41.39 µg/g dust) than on mattresses (median 2.6; maximum 205.03 µg/g dust). On 41/46 carpets (89.1%) and 28/31 mattresses (90.3%), HDM allergen concentrations (Der p 1 + Der f 1) exceeded the value of 0.1 µg/g dust at least once.

Figure 2 shows the variability of Der p 1 concentration at one site within 3–24 months as an example of the variability of both HDM allergens. Table 1 indicates the variability of repeated measurements separately for Der p 1 and Der f 1. The CVs for the sum of allergens (Der p 1 + Der f 1 concentration) were in the same range: carpets, median 75.3 (0–185.9%); mattresses, median 76.6 (0–138.0%). The basic results of the ANOVA (within- and between-site variance and reliability) and the single determination ranges for one measurement are presented in Table 2. Tables 1 and 2 allow a comparison of the variability of Der p 1 and Der f 1 exposure as well as a comparison of concentration and mass determinations.

The 95% CIs for the results of multiple measurements and the number of repeated measurements required to reach a 95% CI of a certain magnitude are summarized in Table 3. The sample sizes for group comparisons necessary to detect a difference of 150, 200, or 400% with 80% power (α = 0.05) are given in Table 4.

The CVs calculated separately for carpet samples from study 1 (residents) and study 2 (trained field workers) did not differ significantly (Table 5). Mattress values could not be evaluated because there were only four sample pairs from mattresses taken in study 2.

Discussion

This study indicates that the variability of HDM allergen concentration at one site in field studies is high. The 95% range of possible values around a single determination's result spans from 5- to 20-fold below to 5- to 20-fold above this value. For the mean of two measurements, the 95% CI is about half as wide. This means that a single measurement does not give precise information about the true exposure over time. Even if two repeated measurements result in a mean value of 1 µg Der f 1/g mattress dust, only concentrations <0.12 µg/g and >8.28 µg/g can be excluded with 95% certainty (corresponding values for Der p 1 0.29 and 3.47 µg/g dust; see Table 3). This variability occurs anywhere in the clinically relevant concentration range above 0.1 µg/g dust.

We have analyzed how much of the observed variability is caused by variability in our method. The CVs for multiple extractions as well as for multiple sampling from one site at the same time did not exceed the variability of the laboratory procedure significantly [intra-assay and interassay CV of maximally 20% in our laboratory and others (*1*)]. The CVs for repeated sampling in the field studies are two- to fourfold higher. We conclude that the additional variability in our field studies must be caused by real variability at the examined homes (changing mite growth conditions, cleaning patterns, visitors, weather-associated dirt transfer).

The design of our study excluded data from a season with higher exposure. We have probably analyzed a data set with lower values and smaller variability compared to data from all seasons. That means the high variability we estimated is probably an underestimation of the real variability of exposure. This variability is considerably higher than that in the Australian study (*8*). This is not explained by the much lower concentrations in our region because our data (Fig. 1) as well as the Australian data demonstrate that the variability of repeated measurements does not depend on their mean result. One more reasonable explanation is that the short time interval between the measurements in Sydney reduced the chance to detect time-dependent variations.

The Australian investigators used a prefilter at the front of the vacuum cleaner to remove coarse particles, whereas in our

Table 2. House dust mite allergen variability between sites and within-site and resulting reliability

	Variance between sites[a]	Within-site variance[a]	Reliability (%)[b]	Single determination range (fold units)[c]
Carpets (n = 46; 120 samples)				
Der p 1 concentration	0.7	0.24	74.4	0.11 ... 9.14
Der f 1 concentration	0.58	0.25	69.4	0.10 ... 9.74
Der p 1 mass	0.8	0.27	74.6	0.09 ... 10.55
Der f 1 mass	0.72	0.27	72.6	0.10 ... 10.46
Mattresses (n = 31; 78 samples)				
Der p 1 concentration	1.00	0.15	86.8	0.17 ... 5.81
Der f 1 concentration	0.83	0.44	65.4	0.05 ... 19.89
Der p 1 mass	0.94	0.29	76.6	0.09 ... 11.20
Der f 1 mass	1.12	0.62	64.5	0.03 ... 34.48

Der p 1, allergen of *Dermatophagoides pteronyssinus*; Der f 1, allergen of *D. farinae*.

[a]Variance components are calculated based on $\log_{10}$ transformed values of Der p1 and Der f 1 in an analysis of variance (ANOVA).

[b]Reliability is calculated as follows: reliability (%) = variance between sites/(variance between sites + within-site variance) * 100.

[c]The single determination range (SDR) is calculated as follows: SDR = 10 $\sqrt{\text{within-site variance}}$ (1.96). SDRs refer to nonlogarithmic Der p 1 and Der f 1 values.

Table 3. Confidence intervals (CIs) for the mean of repeated measurements at the same site and number of measurements required to reach a certain CI[a]

	95% CI in fold units of the mean value		No. of measurements	
	2 Measurements	4 Measurements	CI 0.2- to 5-fold (d = 5)	CI 0.5- to 2-fold (d = 2)
Carpets				
Der p 1 concentration	0.21 ... 4.78	0.33 ... 3.02	2	11
Der f 1 concentration	0.20 ...5.00	0.32 ... 3.12	2	11
Der p 1 mass	0.19 ... 5.29	0.31 ... 3.25	3	12
Der f 1 mass	0.19 ...5.26	0.31 ... 3.23	3	12
Mattresses				
Der p 1 concentration	0.29 ... 3.47	0.71 ... 1.41	2	7
Der f 1 concentration	0.12 ... 8.28	0.22 ... 4.46	4	19
Der p 1 mass	0.18 ... 5.52	0.30 ... 3.35	3	13
Der f 1 mass	0.08 ... 12.23	0.17 ... 5.87	5	27

Der p 1, allergen of *Dermatophagoides pteronyssinus*; Der f 1, allergen of *D. farinae*.

[a]The 95% CI for the mean of n measurements is calculated by dividing the single determination range (SDR) of the measurement by the square root of n. The number (n) of measurements required to reach a 95% CI of a given magnitude (d) is calculated as follows: $n = (1.96 * SDR2)/d^2$.

Table 4. Sample size calculation for comparisons between two independent groups of homes and paired measurements in the same home

	Independent groups of homes: sample size in each group required to detect[a]			Paired measurements: *n* pairs required to detect[a]		
	150% Effect	200% Effect	400% Effect	150% Effect	200% Effect	400% Effect
Carpets						
Der p 1 concentration	476	163	41	241	84	23
Der f 1 concentration	421	144	36	194	68	18
Der p 1 mass	543	186	46	270	94	25
Der f 1 mass	500	171	43	220	77	21
Mattresses						
Der p 1 concentration	583	199	50	220	77	21
Der f 1 concentration	643	220	55	307	106	28
Der p 1 mass	621	212	53	244	85	23
Der f 1 mass	878	300	75	420	145	38

Der p 1, allergen of *Dermatophagoides pteronyssinus*; Der f 1, allergen of *D. farinae*. Sample sizes were determined for given mean differences and the calculated variance components using tables for the two-tailed *t*-test with $\alpha = 0.05$ and $1 - \beta = 0.80$ (*16*).

study samples were extracted unsieved. If allergens attached to bigger particles were subject to greater temporal variability (for example, because of a greater impact of simple cleaning procedures on these particles), the variability of the allergen content of settled dust would be underestimated by examining prefiltered dust. On the other hand, the fine dust fraction may be more relevant for airway exposure. The effect of prefilters on allergen content and particle size in dust samples needs further investigation.

Sampling coarser dust particles did not simply increase variability by dilution of allergen concentrations with varying amounts of allergen-free dirt. Allergen mass tends to vary even more than allergen concentration (Tables 1 and 2). This indicates that, in addition to "dilution" effects by transport of allergen-free dirt into the dwelling, the conditions for allergen production (mite growth) vary significantly even outside the "mite season."

A third possible explanation for the lower variability in the Australian study is that it evaluated repeated baseline measurements in the context of controlled trials of allergen avoidance. It is conceivable that during this period residents treat their carpets and mattresses in a more uniform and standardized way than when not participating in a study investigating primarily allergen exposure.

Der f 1 measurements showed a higher variability than Der p 1 measurements in mattresses but not in carpets. There is no obvious explanation for this observation, and it should be investigated further. Repeated samples from carpets did not show greater variability than samples from mattresses.

We did not find significant differences in the variability of results in study 1 (residents following written instructions) and study 2 (trained field workers). This indicates that dust sampling can be standardized sufficiently by written instructions if one takes into consideration the great overall variability of allergen exposure. This may facilitate future field studies.

Table 5. Variability (coefficient of variation; CV) of house dust mite allergen in dust samples recovered by residents (study 1) or trained field workers (study 2)

	Study 1	Study 2
	Median within-site CV, % (range) (*n* = 24 dwellings)	Median within-site CV, % (range) (*n* = 22 dwellings)
Carpets		
Der p 1 concentration	85.1 (0–129.9)	77.1 (0–132.3)
Der f 1 concentration	78.8 (16.7–139.0)	68.1 (0–140.5)
Der p 1 mass	86.8 (9.3–149.6)	85.9 (5.3–139.0)
Der f 1 mass	72.4 (25.5–138.0)	67.8 (9.6–137.6)

Der p 1, allergen of *Dermatophagoides pteronyssinus*; Der f 1, allergen of *D. farinae*. The significance of the differences between the CVs in study 1 and study 2 was tested with the Mann Whitney *U*-test (significance level $\alpha = 0.05$). There was no significant difference between study 1 and study 2 for any of the four parameters.

The results of this study are especially relevant for studies that compare the effects of interventions or different housing conditions on allergen concentrations in settled dust. Such studies must be performed with sufficient numbers of samples (Tables 5 and 6). Only factors with a strong impact would be detected with small samples. This may be the reason that in some studies the influence of theoretically plausible allergen avoidance measures could not be proven (*17,18*).

Measurments of mite allergen concentrations in settled dust are not a measurement of the allergen entering nose or lungs. HDM allergen concentrations in mattress dust correlate with airborne allergen measured in the close vicinity of the bed (<1 m) but not at a greater distance (*19*). Therefore, concentrations in mattress dust may closely indicate airway exposure during sleep. This may be of clinical relevance for the assessment of the risk of allergic sensitization in early life when the individual spends a great proportion of time in bed. In fact, allergic sensitization to HDM and asthma in 11-year-old children is related to HDM allergen exposure at 1 year of age but not to current exposure (*4*).

During the day, airway exposure is much more determined by activity patterns that influence dust disturbance, vicinity to the floor, or inspiratory flow rate. The variability of allergen concentrations in settled dust may be a minor source of variability compared to these influences. Personal monitoring of allergen entering the airways would considerably improve exposure assessment. Techniques determining nasal HDM allergen exposure as well as the size of the particles on which allergen enters the airways have recently been presented in preliminary reports (*20,21*).

Until such methods are well standardized and easily applicable, the measurement of HDM allergen in settled dust remains the best index of exposure available. Our results indicate that single-point measurements give unreliable information about the long-term allergen exposure of a person. Therefore, the allergen exposure determination should be performed repeatedly in patients at high risk or in studies describing the impact of HDM allergen exposure on health.

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